

THE EFFECT OF ETHANOL EXTRACT OF KEBAR GRASS (*Biophytum petersianum* KLOTZSCH) TOWARDS IMPROVEMENT OF RAT *Rattus norvegicus* LUNG EXPOSED TO CIGARETTE SMOKE

Ishak B. Lekatompessy^{1*}, Ch. D. Umi Bazsary², A. J. A. Unitley²

¹ Student of Biology Department, Faculty of Natural Science and Mathematics, Pattimura University.

² Lecturer of Biology Department, Faculty of Natural Science and Mathematics, Pattimura University.

ARTICLE INFO

Article History:

Received 27th July, 2018

Received in revised form

11th Agustus, 2018

Accepted 03th September, 2018

Published online 30th September,
2018

Key words:

Biophytum petersianum Klotzsch,
Antioxidants, Cigarette smoke,
Lung, Rat.

*Correspondence to Author:

Ishak B. Lekatompessy

Student of Biology Department,

Faculty of Natural Science and

Mathematics, Pattimura University

E-mail:

ishaklekatompessy40@gmail.com

ABSTRACT

Exposed to cigarette smoke is one of the exogenous free radicals, exposure to cigarette smoke it is also associated with stress oxidative. Compounds are able to reduce the free radical in way binding on the free radical known as antioxidants. Kebar grass (*Biophytum petersianum* Klotzsch) is one of the plants that contains of antioxidants, vitamins, and flavonoids that supposedly has ability to fix the lung damage exposed to cigarette smoke. The aim of the research is to know the effect of ethanol extract of Kebar grass (*Biophytum petersianum* Klotzsch) in fixing the rat lung exposed to cigarette smoke. Contains of four treatments and three replicates times. Negative control, positive control, dose 0.0675 and 0.135. The result of the study shows that ethanol extract of Kebar grass able to fix the damage of lung on the ductus alveolaris, sacus alveolaris, and alveoli area that exposed to cigarette smoke. So it can be conclude that ethanol extract of Kebar grass can fix the lung in the form of damage ductus alveolaris, sacus alveolaris and alveoli.

Copyright © 2018, **Ishak B. Lekatompessy**. This is an open access article distributed under the creative commons attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Citation: Ishak B. Lekatompessy^{1*}, Ch. D. Umi Bazsary², A. J. A. Unitley², 2018 "The Effect Of Ethanol Extract Of Kebar Grass (*Biophytum Peterianum* Klotzsch) Towards Improvement Of Rat *Rattus Norvegicus* Lung Exposed To Cigarette Smoke", *International Journal of Health Medicine and Current Research*, 3, (03), 1034-1038.

INTRODUCTION

The Global Youth Tobacco Survey, conducted by the World Health Organization (WHO) in 2000 mention about 20% of students in Jakarta are smokers. And about 30 percent of the population of Indonesia are smokers, with

60 percent of men and 5 percent of women smoke (WHO, 2000). Cigarettes contain more than 4,000 harmful substances including tar, arsenic, formaldehyde, and benzo (a) piren which are carcinogenic. In cigarette smoke also contains carbon monoxide (CO), hydrogen cyanide, nitrogen oxides, and ammonia. In addition free radicals resulting from cigarette smoke led to destruction of alveoli lumen and inflammation of the alveoli that are characterized by increasing number of alveolar macrophages and leukocytes (Manuella et al., 2011).

Free radicals derived from cigarette smoke causes irritation and inflammatory effects (Winarsi, 2007). The effects of free radicals can be prevented by the consumption of food and drink that contains antioxidants counteract free radicals functional especially in the respiratory tract. The body requires extra antioxidants from outside the body such as flavonoids, vitamin E, vitamin C, vitamin A, Se, Zn and glutation. One of the herbs that contain antioxidants and minerals such as vitamin E serves to maintain the integrity of the membrane of the cell, so that the condition of balanced cells to be able to do poliferation and also keep a balance in the cell, a balanced State in the cell will help in the process of metobolisme to do growth. In addition, vitamin E in the body also serves as a antioxidants in the body , the presence of the antioxidant content in the body will be able to reduce the presence of free radicals in the body so as to prevent the occurrence of damage to the cells (Stiphanuk, 2000). In addition there are also flavonoids contained in the Kebar grass already in General acts as an antioxidant. One of the herbs that are known to contain antioxidants that is one of vitamin E that is Kebar grass.

A lot of research that uses Kebar grass more lead to the reproductive system (Unitly, 2013), but there has been no study with Kebar grass to repair the lungs. Kebar grass contain many nutrients and minerals that help in the process of fixing the lung caused by the presence of oxidative stress due to cigarette smoke, in Kebar grass contained vitamin A, vitamin E, vitamin C, flavonoids, lipids and proteins. Vitamin E is very active in preventing the concentration of lipids with the transfer of a hydrogen atom. So, vitamin E removes the radical peroxy faster than the free radical reaction with protein membranes or unsaturated fatty acids, saturated fatty acids do not protect the membrane phospholipids. In addition there are also flavonoids which have been shown to play a role against the hydroxyl radical, superoxide anion radical and peroxy (Latumahina, 2012). This result also complies with the Santos et al., (2004), which States the working mechanisms of flavonoids as giver of Atomic H⁺ and slow down the rate of autooksidasi and inhibit lipid radical formation by providing a hydrogen atom on the lipid radical, then the lipid radical will be transformed into a more stable form and does not result in a heavier damage. This result is also supported by the opinion of Lenzatti, (2010) stating that the compound is effective as an antioxidant flavonoids can through its activities as scavenger and

inhibitor of protease. This will hamper the work of flavonoids of protease to elastic the lungs so that network in the lungs will undergo repairs.

Vitamin C is a water-soluble compound. These compounds, according to (Zakaria *et al.*, 1996), is part of the body's defense system against reactive oxygen compounds in plasma and cells. Vitamin C is capable of a radical reduction of superoxide, hydroxyl, and other reactive oxygen. In addition to the content of vitamin C and vitamin E, Kebar grass also contains lipids that play an important role in the structure and function of cells and proteins are also generally required for the formation of new cells. Whereas vitamins and minerals play a role assisting the process of metabolism of carbohydrates, fats, and proteins in the body (Akhirunnisa, 2010). This became the basis of doing testing of antioxidant activities of ethanol extracts of Kebar grass in mice who are exposed to cigarette smoke. Observation on histology of lungs made in a descriptive way to compare any group of treatment based on the degree of damage with the negative control group (normal group).

METHODS

This research is the research of experimental laboratory, by using the method of RAL (*Complete Random Design*) which consists of four treatments and three times. The animal model used is 12 Sprague Dawley strains of rats which consists of:

P0: The rats not given the extract.

P1: The positive control group exposed to cigarette smoke for 28 days. mice that were not given the treatment.

P2: a group of mice being cigarette smoke for 28 days and given ethanol extracts of Kebar grass 0.0675 mg/head/day for 28 days.

P3: the Group of mice that smoke being exposed for 28 days and given ethanol extracts of Kebar grass 0.135 mg/head/day for 28 days.

In this study the tools and materials used., include the tools used in this research is the animal enclosure experiment, ohaus digital scales, measuring cup, cover glasses, surgical tools, injection spuit animal experiments (skapel, tweezers, scissors, needles, and a table of candles), microtome, micropipet, waterbath , hotplates, blender, wathman filter paper, erlenmeyer flask, rotary evaporator, smoking, smoking chamber pump, microscopes, paper labels, glass and light microscope preparations. The materials used in this research is the rat 12 tails, animal experiments (pellet) cigarettes, aquades, coloring ingredient preparations histology (coloring HE), extract the Kebar grass, ethanol 70%, physiological fluids (NaCl 0.95%) and rice husk. The rat *Rattus norvegicus* was placed on a plastic covered box enclosure wire ram with husk as the pedestal was adapted on the atmosphere of the enclosure for 1 week. After a week later rats presented with 10

cigarettes clove cigarettes, as for a exposed is done twice a day with 5 rods first at 09.00 WIT and 5 second rod at 16.00 WIT. The exposure is done in a special box enclosure in which there are bars (wire delimiter) to separate animals try with the tip of a burning cigarette smoke, so the animals try to directly hit by exposure to the smoke-box treatment features that serve to place the hose contains smoke first accommodated in the process of vacuum exposure done for 28 days.

After that the next process took place with the tinting process using this type of coloring HE (*Hematoxylin eosin*), where the previous animal models have been dissected (necropsy) in advance and taken the organ's lungs. The data obtained are then analyzed in qualitative descriptive by observing the lung histopathology of rats by comparing each treatment.

RESULTS AND DISCUSSION

The result of the Lungs of rat *Rattus norvegicus*

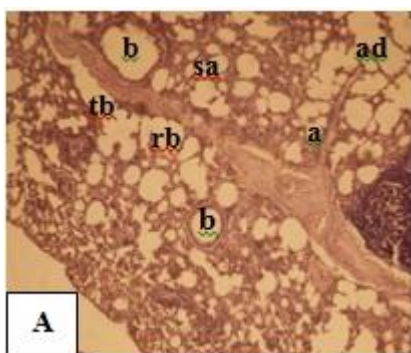


Figure 1A. Negative control group.

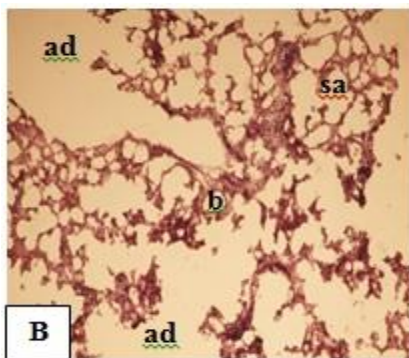


Figure 1B. Positive control group

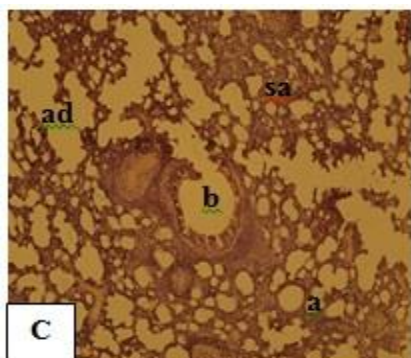


Figure 1C. The granting of ethanol extract of Kebar grass 0.0675 mg/head/day.

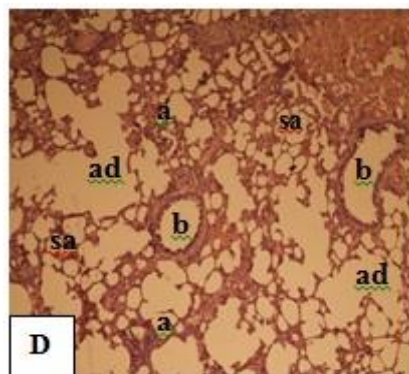


Figure 1D. The granting of ethanol extract of Kebar grass 0.135 mg/head/day .400X magnification. Coloring HE (*Hematoxylin-eosin*).

Descriptions: **b:** Bronchus; **tb:** Bronchi tubule; **rb:** Respiratory bronchi; **da:** Duct alveolaris; **sa:** Sakus alveolaris; **a:** Alveoli.

Damage to The Lungs of Rats Exposed to Cigarette Smoke

The results of the analysis of photomicrograf lung organs of rats with *Hematoxylin eosin* staining (HE) on a group of mice exposed to cigarette smoke for 28 days with 4 animal treatment group test. The negative control group showed lungs seem normal (Figure 1A) is indicated by the presence of terminal bronkiolus, bronkiolus, duct respiratori alveolaris, sacus alveolaris, bronchi and alveoli. Positive control group (Figure 1B) shows lung damage to almost all parts of the lungs that is on the area of ducts alveolary, sacus alveolary and alveoli. A group of ethanol extract of Kebar grass granting doses 0.0675 mg/head/day (Figure 1C) showed the damage to the alveoli seem less visible presence and improvements in the lungs of mice in this group, the presence of visible ductus alveolaris sacus alveolary, bronchi and alveoli, which looks better, picture of lung histology group of mice who were given ethanol extracts of Kebar grass 0.135 mg doses/head/day (Figure 1D) indicates the degree of lung damage that declined with increasing doses of ethanol extract of Kebar grass granting and visible presence of cell regeneration and have a picture that is almost the same marked with the negative control group the presence of bronchial, duct alveolary, sacus alveolary and alveoli and a large bronchus. Damage in the form of destruction of alveoli lumen i.e. damage to the alveolar walls which damage epithelial membrane protein elastin and experiencing thinning of atrophy (Maria & Irawati, 2010). This situation occurs due to free radicals contained in cigarette smoke triggered an increase in neutrophils and sekuestrasi inactivation of α 1-AT IE α 1-AT IE a protein that plays a role in inflammatory processes in the cells, this protein deficiency causes tissue damage alveoli so that the process of circulation of O₂ and CO₂

is not running normally. Free radicals attack cell membrane causing the structure of the cell membrane is damaged so that free radicals can easily enter into the cytoplasm and finally damage the cell nucleus.

Free radicals contained in cigarettes can inhibit acetylcholinesterase, thus causing the accumulation of kolin in the lungs. This accumulation of acetylcholine stimulates bronchial to contribute so that the destruction of the walls of the alveoli occurs. The hydroxyl radical (OH[•]), radical superoxide (O₂^{•-}) and hydrogen peroxide (H₂O₂) in the body can harm the polyunsaturated fatty acid on the cell membrane and also potentially damaging to DNA bases so as to disrupt the genetic information system (Halliwell & Gutteridge, 1991).

The study results indicate the occurrence of damage to the alveoli of the lung damage the ducts alveolary, sacus alveolary and alveoli of the lungs of rats (Figure 1B). This caused oxidative stress occurs continuously lead to the occurrence of the damage to the walls of the alveoli it is because of the cigarette smoke can inhibit acetylcholinesterase, causing the accumulation of colin in the lungs. This accumulation of acetylcholine stimulates bronchial to contribute so that the destruction of the walls of the alveoli occurs (Al Idrus *et al.*, 2016).

Repair of Rat Lung Cells Exposed to Cigarette Smoke of Awarding Post-Graduate of Kebar Grass Grating

The image of the lung photomicrograf the Group of mice given ethanol extract of Kebar grass with a dose 0.0675 mg/head/day (Figure 6C) showed the damage to the alveoli seem less visible presence and improvements in the lungs of mice in this group visible presence of duct alveolary, sacus alveolary, bronchi and alveoli, which looks better, decreasing damage to alveoli allegedly due to binding of free radicals by antioxidants and mineral content contained in the ethanol extract of Kebar grass (*Biophytum petersianum* Klotzsch). The image of the lung histology group of mice who were given ethanol extracts of Kebar grass 0.135 mg doses/head/day (Figure 1D) indicates the degree of lung damage that declined with increasing doses of ethanol extract of Kebar grass given and visible presence of cell regeneration and have almost the same histology picture with negative control groups are characterized by the presence of bronchial, duct alveolary, sacus alveolary and alveoli and a large bronchus. These results are in line with the Lenzatti, (2011) the regeneration of the cells of the lung characterized by increasing the activity of the protease inhibitors in inhibiting the release of TNF- α -like cytokinin, IL-1beta, IL-2 and interferon-gamma which helps in the lungs so that the tissue on the lungs will undergo repair of destruction at walls of the alveoli become better. On the research group's treatment with doses of extract 0135 mg/head/day gives the role of protective of cell membranes are better because of the concentration of antioxidant compounds are more so that it can be balanced the production of free radicals and

stop the chain reaction due to exposure to cigarette smoke, so that the activity of neutrophils can be offset by antiprotease. Vitamin E in Kebar grass was very active in preventing the concentration of lipids with the transfer of a hydrogen atom. So, vitamin E removes the radical peroksil faster than the free radical reaction than the free radical reaction in addition to Vitamin E contained also flavonoids. Flavonoids play a role against the hydroxyl radical, superoxide anion radical and peroksil (Latumahina, 2012). This result also complies with (Santos *et al.*, 2004). mechanism of action of flavonoids as giver of H⁺ atom and slows the rate of autooksidative and inhibit lipid radical formation by providing a hydrogen atom on the lipid radical, then the lipid radical will be transformed into a more stable form and does not result in a heavier damage.

In addition Vitamin C in Kebar grass can reduces superoxide radicals capable of Kebar grass, hydroxyl, and other reactive oxygen. In addition to the content of vitamin C and vitamin E, flavonoids and Kebar grass also contains lipids that play an important role in the structure and function of cells and proteins are also generally required for the formation of new cells. Whereas vitamins and minerals play a role assisting the process of metabolism of carbohydrates, lipids, and proteins in the body (Akhirunnisa, 2010). So based on the results of the study it can be concluded that Kebar grass (*Biophytum petersianum* Klotzsch), have the potential to improve damage lung cells due to exposure to cigarette smoke.

REFERENCES

1. Akhirunnisa D.V. 2010. Hepatoprotective effect of ethanol Extract 50% Fungus Lingzhi (*Ganoderma lucidum*) on Male Rats induced by Paracetamol. Thesis. Faculty Of Pharmacy. Muhammadiyah University Of Surakarta. Surakarta.
2. Alwi I., S. J., H. G., K. Juferdy, and T.D. Luis. 2016. Management in the field of Pathology In Clinical Practice Guidelines. International Publishing. Jakarta.
3. American Thoracic Society. 2005. American Thoracic Society For Management Of Chronic Obstructive Pulmonary Disesase. New York 2 (14): 3-6.
4. Halliwell B and Gutteridge, M.C. 1991. Free Radicals in Biology and Medicine. 2nd Edition. Oxford 310-314 Thing. New York.
5. Latumahina G. 2012. The role of the Honey as antioxidants in preventing Pancreatic Damage House mouse (*Mus musculus*) exposed to Smoke Cigarettes. Thesis. Biology Majors. Faculty of mathematics and natural sciences. Pattimura University. Ambon.
6. Lenzatti M., A. Lopes, T.S. Ferreira., R. S. de Moura, a. Resende, L.C. Porto, and S.S. Valenc. 2011. The Mate Tea Ameliorates Emphysema In

Cigarette Smoke-Exposed Mice. Experimental lung research 8 (4): 246-257.

7. Mary and Irawati. 2010. Management of

Pulmonary Edema on the case of VSD and species of VAP. Anesthesia & critical care 3(5): 53-60.
